

## DISEASE NOTES



# Thousand Cankers Disease Caused by *Geosmithia morbida* and Its Insect Vector *Pityophthorus juglandis* First Reported on *Juglans nigra* in Tuscany, Central Italy

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Thousand cankers disease (TCD) is a disease complex originating in the mid-1990s in the western United States, where it affects some native walnut species, but is particularly aggressive on black walnut (*Juglans nigra* L.) (Kolařík et al. 2011; Utley et al. 2013). TCD is caused by the combined action of the fungus *Geosmithia morbida* M. Kolařík, E. Freeland, C. Utley & N. Tisserat (Ascomycota, Hypocreales) and its vector, the walnut twig beetle (WTB) *Pityophthorus juglandis* Blackman (Coleoptera, Curculionidae). *G. morbida* develops around the beetle galleries, causing numerous cankers, and eventually tree mortality. In Europe, TCD was first reported in 2013, on black walnuts from the Veneto region, in northeastern Italy (Montecchio et al. 2014a); later, both organisms, or the vector alone, were reported from other regions of northern Italy (Montecchio et al. 2014b). As part of a monitoring program to detect the occurrence of TCD in Tuscany, eight-unit Lindgren multiple funnel traps (Contech Enterprises, Victoria, BC, Canada) were deployed in April 2018 in various walnut plantations. Two traps baited with WTB (Alpha Scents, Portland, OR) were set up at

each site and inspected at 15-day intervals. From the first inspection date, bark beetles were found trapped in a plantation (about 6,000 m<sup>2</sup>) of *J. nigra* with scattered individuals of *J. regia* L. at Rosano (43°46' N, 11°24' E, about 100 m above sea level) and morphologically identified as WTB (Wood 1982). Following this discovery, all trees in the stand were carefully inspected for TCD symptoms and WTB entry and exit holes. Branches with wilt and the typical flagging of TCD, and with WTB holes, were found on *J. nigra* only. Galleries, adults, and larvae of WTB were observed under the bark. Galleries were densely covered with the characteristic whitish *G. morbida* mycelium consisting of long, verticillate conidiophores producing yellowish conidia with an average size of 5.5 × 2 μm. *G. morbida* was isolated on potato dextrose agar from infected wood, and from WTB adults and larvae. Both the fungus and the insects were identified at the molecular level. The ITS1-5.8S-ITS2 rDNA region of *G. morbida* was polymerase chain reaction–amplified with ITS4 and ITS6 universal primers, and a portion of the *CoxI* mitochondrial gene of *P. juglandis* was amplified with LCO1490 and HCO2198 primers (Folmer et al. 1994). Amplicons of both organisms were purified and sequenced (GenBank accession nos. MH620784 [*G. morbida*] and MH666050 [*P. juglandis*]). A BLAST search of the ITS and *CoxI* sequences with the closest sequences deposited in the database revealed a 99% identity of the fungus with the North American and Italian isolates. Sequences of the insect displayed 100% identity with specimens from the United States and Piedmont (Italy). This is the southernmost finding of TCD in Europe. The disease is of particular concern in Italy because of the high economic value of walnut fruit and wood. Strict phytosanitary inspection of the wood of *Juglans* and *Pterocarya*, of untreated wood packaging material, and of plants for planting is essential at all ports of entry to prevent any further introduction of TCD into this country. At the same time, a ready and thorough monitoring of walnut trees and plantations is necessary to promptly detect and eradicate any further outbreaks. These are the only options available to stop further spread of TCD southward, where walnut cultivation is of prime importance.

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